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# Morphology and Morphometrics of *Heterodera filipjevi* (Madzhidov, 1981) Steller, 1984 From Kohgiluyeh and Boyer-Ahmad Province, Iran

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**Abstract:** During 2007 and 2008, soil samples of wheat and barley fields of the cold regions of the Kohgiluyeh and Boyer-Ahmad province were surveyed for cereal cyst nematode. In 65% of samples cysts were seen. Morphological and morphometrics of cysts, cone top structure and second stage juveniles of the isolated populations were studied. Detailed taxonomic studies revealed the existence of *H. filipjevi*. This is the first report of the species in the province.

**Key words:** Yasouj, wheat, barley, avenae group, cereal cyst nematode, morphometric

## INTRODUCTION

Cereals, such as wheat, oats and barley, are among the major staple crops of economic importance worldwide. These crops are parasitized by many pathogens and pests including plant parasitic nematodes. Among nematodes, cyst-forming nematodes (*Heterodera* spp.) are considered to be very damaging because of crop losses they induce and their worldwide distribution. Currently, the genus *Heterodera* contains more than 60 species. The cereal cyst nematodes, *Heterodera* spp., are major pests of cereal crops and their worldwide occurrence has been known for many years (Greco *et al.*, 2002). Wheat is the major field crop in Kohgiluye and Boyer-Ahmad province with 282 thousand metric ton produced annually on 205 thousand ha (MJA, 2008).

A survey of cereal cyst nematodes carried out in cereal fields of the Kohgiluye and Boyer-Ahmad province to clarify the presence of the any cyst nematode in these fields. Based on comparative morphological and morphometrical studies on cysts and second-stage juveniles, the collected populations were identified and the results are demonstrated in this study.

## MATERIALS AND METHODS

During the course of investigation to survey the occurrence of cereal cyst nematode in the cold regions of the Kohgiluyeh and Boyer-Ahmad province, soil samples (500 g) were collected from the rhizosphere of wheat and barley of several fields of seven wheat and barley growing districts namely Yasouj, Pataveh, Kakan, Tang-e-Sorkh, Chitab, Sepidar and Dasht-e-Rome, 15 days before harvest (Fig. 1). The soil samples were processed using Cobb's sieving technique (Cobb, 1918). Twenty and 100 mesh sieves were used for washing the soil. The cysts were

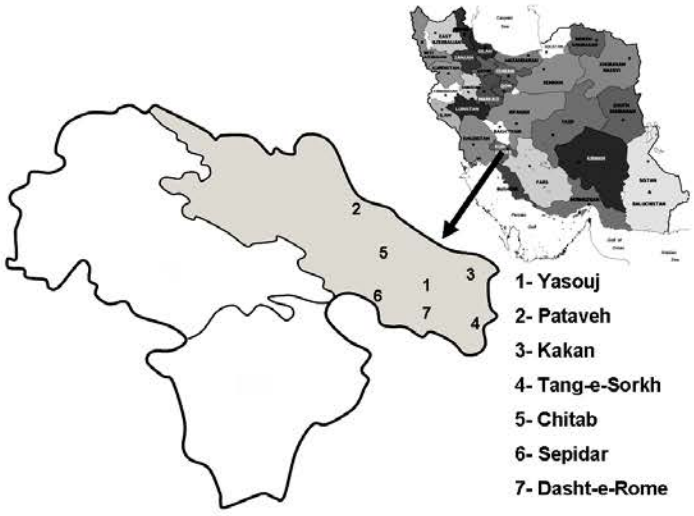


Fig. 1: Localities from where populations of *Heterodera filipjevi* were collected

collected and then processed as follows for detailed morphological studies.

The cone tops of cysts were mounted in Canada balsam (Kornobis, 1976). Few mature cysts from each population were placed in fresh water and the second stage juveniles (J2s) were allowed to emerge. After about a week the J2s were picked from the suspension and concentrated. The concentrated suspensions were killed in hot water bath and fixed in 2% formaldehyde. J2s were picked from the respective fixed nematode suspensions. Fixed juveniles were processed in glycerin and their permanent mounts were prepared in dehydrated glycerin.

Cyst, cone top structure and J2s morphometric means including cyst length, cyst width, cyst length to cyst width ratio, vulval slit length, length of fenestra, breadth of fenestra, fenestral length to fenestral breadth ratio, vulval bridge length, vulval bridge breadth, under bridge length, under bridge breadth, J2s body length, maximum body width, a ratio, distance from head to oesophageal gland lobe, b ratio, stylet length, tail length, C ratio and hyaline tail length were measured using the compound research microscope, Sa Iran, having a drawing tube attachment (Table 1 and 2, Fig. 2).

Table 1: Measurements of J2s of *Heterodera filipjevi* from different localities [Mean; (Range ) in µm, except ratios]

| | | | Holgado *et al.* (2004b) | | Subbotin *et al.* (2003) | |
|---|---|---|---|---|---|---|
| Character | K.B. Province, Iran | Madzhidov, 1981 Tadzhikistan | Norway 184 | Norway 185 | UK | Turkey |
| Body length | 543.16 | 510 | 520 | 490 | 522 | 543 |
| | (490-570) | (430-580) | (490-557) | (455-532) | (488-558) | (494-592) |
| Body width | 20.72 | 22.9 | 19.8 | 19.5 | -- | -- |
| | (20-23) | (21-24.5) | (19.5-21.5) | (18-22) | | |
| a ratio | 26.25 | 23.6 | 26.2 | 25.1 | 25 | 26 |
| | (24.5-28.25) | (21-25) | (24-29) | (22.5-29) | (23-27) | (23-28) |
| Head to Oes. | 119.52 | -- | -- | -- | -- | -- |
| Gland lobe | (112-128) | | | | | |
| b ratio | 4.55 | -- | -- | -- | 4.3 | 4.1 |
| | (4.02-4.96) | | | | (4-4.7) | (3.8-4.4) |
| c ratio | 8.78 | -- | -- | -- | 8.8 | 8.8 |
| | (7.97-9.52) | | | | (8.2-9.4) | (7.7-9.5) |
| Stylet length | 23.16 | 26.5 | 24.1 | 23.3 | 24 | 25 |
| | (22-25) | (21.7-30.8) | (22.3-25.5) | (22-24.5) | (24-26) | (25-27) |
| Tail length | 61.96 | 51 | 59.3 | 57.5 | 59 | 62 |
| | (56-66) | (49-63) | (54.5-67.5) | (52-60) | (53-64) | (54-67) |
| Hyaline tail length | 33.64 | 34.8 | 36 | 35 | 35 | 37 |
| | (30-38) | (31-39) | (31-41) | (30.5-41) | (29-39) | (32-45) |

Table 2: Measurements of cyst and cone top of *Heterodera filipjevi* from different localities [Mean; (Range) in µm, except ratios]

| | | | Holgado *et al.* (2004) | | Subbotin *et al.* (2003) | |
|---|---|---|---|---|---|---|
| Character | K.B. Province, Iran | Madzhidov, 1981 Tadzhikistan | Norway 184 | Norway 185 | UK | Turkey |
| Length | 692.76 | 690 | 680 | 692 | 796 | 786 |
| | (520-880) | (490-830) | (458-874) | (455-869) | (696-936) | (672-888) |
| Cyst | 499.24 | 490 | 518 | 509 | 592 | 562 |
| Width | (350-650) | (340-620) | (306-747) | (253-657) | (528-672) | (480-600) |
| Cyst L/W | 1.39 | 1.4 | 1.3 | 1.4 | 1.3 | 1.4 |
| Ratio | (1.14-1.62) | (1.1-1.6) | (1-1.6) | (1-1.8) | (1.3-1.4) | (1.2-1.5) |
| Vulval slit | 8.18 | 7.3 | 8.7 | 7.9 | 9.3 | 9.5 |
| Length | (7-11.5) | (6.3-8.4) | (7.6-10.8) | (6-9.3) | (7.8-12) | (7.8-12) |
| Fenestral | 51 | 51.5 | 48.1 | 47.8 | 54 | 59 |
| Length | (40-60) | (41-64) | (38.4-58.4) | (38.6-56.2) | (47-62) | (54-66) |
| Semifenestral | 25.36 | 27.5 | 25.3 | 23.3 | 29 | 28 |
| Breadth | (20-31) | (21-33) | (20.8-30.8) | (19.3-32) | (23-31) | (27-31) |
| Fenestral | 2.05 | -- | 1.9 | 2 | -- | -- |
| L/B ratio | (1.68-2.65) | | (1.7-2.1) | (1.7-2.8) | | |
| Vulval bridge length | 9.97 | 7.7 | 10.2 | 10.9 | -- | -- |
| | (7-12) | (6.3-9.4) | (7.2-12.4) | (8-13.1) | | |
| Vulval bridge | 9.08 | 8 | -- | -- | 13 | 12 |
| Width | (6-11) | (6-9) | | | (12-16) | (12-14) |
| Underbridge | 75.52 | 82.4 | 70 | 82 | 74 | 76 |
| Length | (65-91) | (72.5-101.5) | (53-85) | (60-110) | (70-78) | (66-93) |
| Underbridge | 7.48 | 16.8 | 7.7 | 6.5 | -- | -- |
| Width | (6-10) | (15.4-17.5) | (5.7-11.3) | (4-9) | | |

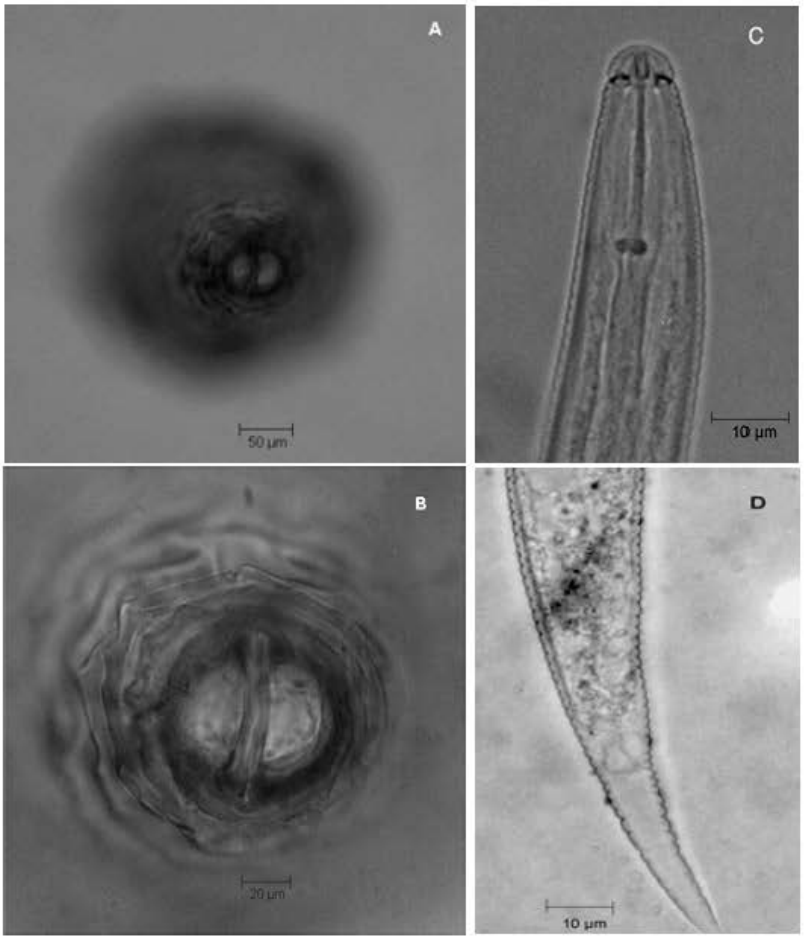


Fig. 2: Morphology of *Heterodera filipjevi*. (A) The bifenestrate vulval cone, (B) The distinct underbridge, (C) The anterior part of the second stage juvenile with the robust stylet having anteriorly concave knobs and (D) Tail of the second stage juvenile

## RESULTS AND DISCUSSION

Morphological and morphometrical studies on the populations showed that cyst and juvenile characters closely agree with those of *H. filipjevi* from Tadzikistan. Measurements of cysts and second stage juveniles are presented in Table 1 and 2. The second stage juvenile body length varies between 490-570 µm and width is 20-23 µm. The stylet is robust and measures 22-25 µm with moderately concave knobs. Cysts are lemon-shaped, partially transparent, light brown in color and having ridges running in zigzag patterns. The cyst length and width are 520-880 and 350-650 µm, respectively. The vulval cone is bifenestrate with an underbridge varies 65-91 µm in length and 6-10 µm in width.

The *H. avenae* group contains 12 valid cyst-forming species with the pronounced vulval cone, a bifenestrate configuration of the vulval area and a vulval slit length less than the width of the fenestrae (Handoo, 2002). The most economically important cereal cyst nematode species damaging winter cereals are: *Heterodera avenae*, *H. hordecalis* and *H. filipjevi* which most often confused with *H. avenae* (Greco *et al*., 2002). The cereal cyst nematode, *Heterodera avenae* is the principal species on temperate cereals, while another important cereal species, *H. latipons*, is essentially only Mediterranean in distribution, being found in Syria, Israel, Cyprus, Turkey, Italy and Libya. However, it is also known to occur in northern Europe. Another species with an increasingly wide distribution is *H. filipjevi*, formerly known as Gotland strain of *H. avenae*. It has been

found in Tadzikistan, Russia, Iran, India, Sweden and Turkey (McDonald and Nicol, 2005).

In 1964 a cyst-forming nematode identified as *H. avenae* was found in Tadzikistan and was subsequently described as a new species, *H. filipjevi*, by Madzhidov (1981) on the presence of a characteristic underbridge in the cysts and differences in morphometrics of juveniles and cysts from other species. It has also demonstrated that the species is widely distributed in the former USSR and also reported from Uzbekistan, Ukraine, Bulgaria, Germany, Sweden, England, Poland, Estonia, Spain (Subbotin *et al*., 2003), Norway (Holgado *et al*., 2004a, b) and Slovak (Renco, 2005).

Presence of *H. avenae* group in many regions of Iran has been reported by several workers (Sturhan, 1996). Sturhan (1996) has been reported the occurrence of *H. filipjevi* in Iran. He has been concluded that besides *H. filipjevi* and *H. latipons*, more species of the *H. avenae* group occur in Iran and as long as there is no evidence that the characters were used in identification of the above mentioned species, are in fact *H. filipjevi*. This species can be separated from other species of *H. avenae* group on the basis of morphology of the cyst, cone top and second stage juveniles (Handoo, 2002). Based on morphometrics of the second stage juveniles, cysts and cone top structures, the isolated populations were identified as *H. filipjevi*. The studies have shown that *H. filipjevi* is widespread in many other Mediterranean regions and it can be concluded that the main area of distribution of is in the east-European and Oriental region (Rumpenhorst *et al*., 1996). According to Damadzadeh and Ansaripour (2001) in 20% of samples the cysts of *H. filipjevi* were seen. However, complementary studies are required to survey the distribution of the species in the other regions of Iran.

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